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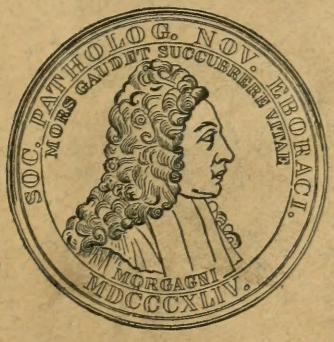

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DR. HORST OERTEL, *President.*

REPORT OF TWO CASES OF ADDISON'S DISEASE.

B. C. CROWELL, M.D.

The widespread interest in Addison's disease at the present time perhaps justifies the report of two cases presenting many interesting features.

My first case is that of an Italian married woman, thirty-nine years of age, a shopworker, who was under observation for only forty-eight hours before death. The history was that of good health until three months previous to admission to the hospital. She then had severe pain in the abdomen and back, which for a time became less severe, and was accompanied by sleeplessness and loss of appetite and strength. After two

months her weakness became so severe as to confine her to bed; at this time she had abdominal pain, vomiting, hiccough, insomnia, constipation, and headache, and in the last three months she had lost thirty pounds in weight. Her hands and face grew darker during the last month.

While under observation the essential features which were noted clinically were pigmentation (to be described in the autopsy protocol), marked asthenia, weak pulse (B. P. 84 mm. Hg), and signs of apical tuberculosis. She had an antemortem rise of temperature to 104.2° F., and died with pulmonary edema.

The autopsy was performed thirteen hours post mortem, and was limited to an abdominal incision, through which I was able to remove the thoracic viscera. Externally she was a well nourished woman with brownish pigmentation of the face, back of hands, lips, and gums. She presented the external features indicating status lymphaticus, with the single exception that the hair was more abundant than would be expected in this type in a woman of her age.

The gross anatomical diagnosis was: Chronic fibro-caseous tuberculosis of both adrenals; pigmentation of skin; chronic adhesive pleurisy and pericarditis; healed apical pulmonary tuberculosis; healed tuberculosis of bronchial and retroperitoneal lymph nodes; lymphoid hyperplasia of stomach and intestines; chronic parenchymatous nephritis; chronic salpingitis and ovaritis. No accessory adrenals and no thymus gland were found.

This diagnosis was supplemented microscopically as follows: Tuberculosis of thyroid (weight, 35 grams); tubercles in liver and spleen; hypertrophy of islands of Langerhans (weight of pancreas, 75 grams). The left adrenal was small and very firm, and on section none of the normal markings of the gland could be distinguished, the gland substance being completely replaced by large, white, partly caseous masses. The right adrenal was considerably enlarged, its surface being pale and nodular. On section, there were seen few remains of the markings of the gland, the cortex being represented by faint yellowish

streaks. At the lower pole was a large caseous focus, and surrounding the entire cortex, for a width greater than that of the normal gland, there was pale firm tissue containing a few caseous foci. Microscopically, the adrenals showed complete destruction, being entirely replaced by a central caseous area surrounded by tuberculous granulation tissue, containing some tubercles and areas filled with plasma cells.

The second case was that of a Canadian farmer, thirty-four years of age, who at three years of age had a lesion which was represented by a complete ankylosis of the left hip. He had had bronzing of the skin for eleven years, and had lost twenty-five pounds in weight in the three months previous to entrance to the hospital, where he sought admission on account of weakness, soreness of the muscles, and loss of appetite. Aside from his ankylosed hip, pigmentation (to be described later), asthenia, soreness of the muscles, and low blood pressure (85 mm. Hg), nothing remarkable was noted clinically.

At the autopsy, which was performed two and a half days post mortem, he was found to be still well nourished, with marked bronzing of the skin of the head, face, and dorsum of the hands, and pigmentation of the lips, buccal mucosa, and glans penis. He also presented numerous symmetrically distributed, circular, macular lesions, about 7 mm. in diameter, some with pigmented margins, over the trunk and extremities, in front and behind.

The gross anatomical diagnosis was: Caseous tuberculosis of adrenal glands; chronic coxitis (tuberculous ?); chronic adhesive pleurisy; chronic ossifying myositis of gluteus medius (left); hyperplasia of lymphoid tissue of lingual and pharyngeal tonsils, pharynx, larynx, spleen, and small and large intestines; congestion of lungs; status lymphaticus. No accessory adrenals were found. There was no arterial hypoplasia.

Microscopically, the adrenals showed the same complete fibro-caseous destruction. The semilunar ganglia were large and contained chromaffin: There were some small cervical nodes containing healed tuberculous areas. The pancreas weighed

sixty-five grams, and showed no pathological change; nor did the thyroid, which weighed forty-five grams. The thymus, which weighed twenty-one and a half grams, showed marked hyperplastic changes. One parathyroid, which was sectioned, was found to be the seat of a very extensive fibrosis showing only small remnants of the gland embedded in the fibrous mass. This is a condition which I have not seen described elsewhere.

A third case occurring in our laboratory some time ago, and already described by Dr. Pappenheimer in connection with status lymphaticus, was that of a woman of thirty-two years, who had a typical clinical history of Addison's disease. She presented at autopsy: Diffuse bronzing of the skin; pigmentation of mucous membranes; tuberculosis of both adrenals with complete destruction of the medulla and only microscopic rests of the cortical substance; tuberculous spondylitis; general lymphoid hyperplasia; arterial hypoplasia; a ten gram thymus.

It is interesting to note that these three cases all show signs of a status lymphaticus, in view of Hedinger's published observations of seven out of fifteen cases of Addison's disease associated with status. Hart and Wiesel have also emphasized this coincidence, and Wiesel reports a case of an eighteen-year-old man who died while swimming, and who was found to have a large thymus and an hypoplasia of the chromaffin system. To the latter Wiesel ascribes his sudden death, rather than to his status lymphaticus.

As to the causation of Addison's disease, about the only generally accepted theory is that it is a disturbance in the chromaffin system, of which the adrenal is supposedly the main depot. Addison's original tenet, that tuberculosis of the adrenals covers all, has been found wanting.

Lewin in the study of 370 cases of typical Addison's disease found:

Typical cases of Addison's with sound adrenals.....12 per cent.
Typical cases of Addison's with diseased adrenals.....88 per cent.
Diseased adrenals without bronzing.....28 per cent.
Diseased adrenals with bronzing.....72 per cent.

He also mentions forty-four cases of destruction of the adrenals by various diseases in patients dying of intercurrent affections who had had no evidence of Addison's disease during life.

That even tuberculosis of the adrenals does not always cause Addison's disease is shown by numerous cases, as, for example, a case reported by Wiesel of a sixteen-year-old girl who had no symptoms of Addison's in spite of extensive tuberculosis of adrenals, kidney, bladder, and genitalia, and abundant chromaffin cells in the solar, suprarenal, and hypogastric plexuses, and in the ganglia along the vertebral column. Wiesel holds that Addison's disease is a disease of the chromaffin system and not of the adrenals alone; while Karakascheff holds that the disease is due to destruction of the cortex of the adrenal. He reports a case of complete destruction of the medulla by an old hemorrhage without Addison's.

At the present time these seem to be the two main views in reference to Addison's disease. Karakascheff is very strongly of the opinion that the cortex of the adrenals can not be destroyed without producing Addison's, and explains reported cases on the ground of accessory adrenals composed of cortical tissue. He also reports some interesting cases of Addison's disease following destruction of the adrenals sequent to thrombosis of the adrenal veins.

The question, to what extent the extra-adrenal part of the chromaffin system can compensate for entire destruction of the adrenal part, is one difficult to settle, as the bounds of the chromaffin system are being constantly extended by different observers, and some of the parts already described are somewhat inaccessible in the ordinary autopsy dissection. Recently the heart and ovaries have been said to contain chromaffin cells. But it may perhaps be open to question also whether all of our staining reactions are really specific; that is, whether there are not some chemically similar substances which give the same reactions.

There have been several physiological methods devised for determining the presence of adrenalin.

1. The first and best known of these is that of Oliver and Schaeffer, who first described the angiotonic effects of extracts of the gland on intravenous injection.

2. Ehrmann found that the pupil of the excised frog's eye dilates in the presence of adrenalin in dilutions as high as 1 in 10,000,000.

3. Meyer's reaction is dependent upon the contraction of portions of excised vessels when subjected to adrenalin.

4. Lastly, Comessatti has described a colorimetric reaction when adrenalin and mercuric chloride are mixed in certain proportions.

Oliver and Schaeffer found the extracts of the adrenals in two cases of Addison's disease inactive as far as the blood pressure raising reaction was concerned; and in the September number of the *Archives of Internal Medicine*, Wells and Greer have shown the extracts of two hypernephromata to be inactive by the first, second, and fourth tests.

Using the method described by Wells and Greer, I made extracts of the adrenals of my second case, and found that intravenous injections of 2 c. c. produced no rise in blood pressure in the rabbit—this being tried with each adrenal mass independently—while an extract of a healthy adrenal obtained four and one-half days post mortem, and prepared in exactly the same way, produced a distinct sharp rise when 0.5 c. c. was injected.

To show that adrenalin if present in the caseous adrenals could not have been masked in this experiment by a possible depressor action of any of the products of caseation, I injected a rabbit intravenously with a similarly prepared extract of some caseous mesenteric glands obtained from a tuberculous infant and found no change in blood pressure produced.

Similarly, the extract of the caseous adrenals produced no dilatation of the excised frog's eye in several hours, while the healthy adrenal extract produced temporary dilatation as did adrenalin chloride in 1 in 10,000 solution.

W. H. Schultz, of the Hygienic Laboratory of the Public

Health and Marine-Hospital Service, has recently said that naked eye readings of the pupil are of little value, and has devised an elaborate apparatus for observing the pupil and making accurate measurements under constant temperature and light. But I think my observations are not entirely devoid of value, as they were made simultaneously under identical conditions of temperature and light, and the independent readings of four observers coincided.

The solution of the cause of Addison's disease will probably not precede a much more accurate knowledge of the interrelations of the various ductless glands, suggestions of which are coming from the physiologists. Falta conceives of the adrenals as being active in the mobilization of carbohydrates, which are oxidized by the pancreatic secretions, and believes that in many cases of diabetes, besides an insufficiency of the pancreas there exists a primary increase of carbohydrate mobilization by means of a hyperfunction of the chromaffin system. Thus, the adrenals and pancreas are related. The thyroids stimulate the sympathetics, which form a part of the chromaffin system, and the parathyroids depress them. Thyroidectomy in dogs prevents adrenalin glycosuria and diminishes the angiotonic effect of adrenalin, while thyreoparathyroidectomy increases the glycosuria.

The relation between the hypophysis and the genital organs is well recognized, and pancreatic disturbance, manifested by diabetes, is not infrequent in acromegaly, which is probably due to a hyperfunction of the hypophysis.

In my cases there are many factors to consider. In the first, we have found destruction of the adrenals, partial thyroid destruction by tuberculosis, hyperplasia of the thymus, and hypertrophy of the islands of Langerhans. In the second case, aside from the adrenal destruction, we have the thymus and the destroyed parathyroid.

What relations, if any, these bear to one another, and their exact bearing on the clinical course, it is beyond the province of this paper to discuss.

Discussion.

Dr. Charles Norris said that he would like to add a few words upon the intimate connection which evidently exists between status lymphaticus and the diseases of the ductless glands, such as acromegaly, Basedow's disease, and, as had just been shown, Addison's disease. His reason for taking up the subject of status lymphaticus was that in the cases of Addison's disease which he had seen there had been an association with status lymphaticus; but it was a well known fact that the static condition in Addison's was not nearly so marked as it was in the average case of Basedow's, where the condition was usually extremely well marked. He thought that the actual fact was that if the term status were extended to include all cases showing slight or partial lymphoid hyperplasia, it would be found that all Addison's cases had developed in subjects having the lymphatic constitution. He had used the term recessive to designate cases in which the lymphatic hyperplasia was not abundant. In well marked cases of status lymphaticus, the lymphoid hyperplasia was present throughout—in the spleen, the lymph nodes, especially the mesenteric, Peyer's patches, etc. It was his conception of the status lymphaticus that people were born with the static constitution. In this he was borne out by Martels who had dropped the term status lymphaticus and used the term hypoplastic constitution. The recessive theory was beautifully demonstrated in the Peyer's patches, especially in those of adults. In such cases one saw irregular hypoplasia. There were in parts of the patch, tumor-like masses; in other parts, homogeneous scar-like tissue. These areas of scar tissue were found to be made up of hyalin connective tissue. The same thing occurred in the follicles of the spleen, which were transformed into fibrous tissue. As to the external appearance of status cases, Dr. Norris spoke only briefly, but he considered that this lent weight to his theory of a constitutional anomaly. In well marked cases the diagnosis was easy to make. Another thing which confirmed him in his belief that the status was a constitutional anomaly was the association of the status with hypoplasia of the arterial system and of the genital organs.

TERATOMA TESTIS.

JAMES EWING, M.D.

In the text books on surgery and pathology, one finds under the heading of tumors of the testicle a considerable list, including fibroma, myxoma, myoma, chondroma, sarcoma, lymphadenoma, carcinoma, teratoma, and mixed tumors. This classification is based largely on the work of Langhans, who was one of the first to classify testicular tumors according to microscopical structures. He recognized most of these classes as separate, but one of his contributions to the subject was the identification of the so-called alveolar sarcoma of earlier writers with carcinoma.

Wilms, in 1896, took up this subject and concluded that there was a close relationship between several of these tumors. Wilms examined various types of mixed tumors and found that they were all tridermal in structure, and he claimed that all such tumors had an identical pathogenesis. He recognized in many of the teratomata, areas of carcinoma, sarcoma, chondroma, and myoma, and he observed also in some instances that the main bulk of the tumor might be composed of any one of these elements. He even suggested that all tumors of the testicle composed exclusively of one of these elements might be of teratomatous origin; but he declined to assert that such was the case and accepted two definite classes of testicular tumors: one derived from teratoma, and one from the adult elements of the testicle. However, he recognized the indications which the study of teratomata gave as to the possible identity of most of the tumors of the testicle. Since Wilms' time further study of the groups has shown one important principle which Pick has emphasized strongly: that is, the tendency toward the preponderance of one element in teratomata. In the case of the myomata especially, suspicion was early aroused that rhabdomyoma might represent a one-sided development of a teratoma, and Ribbert showed from his study of three cases of rhabdomyoma that they were all portions of teratomata in which the other ele-

ments aside from the muscle had been suppressed. In the same way it has been generally agreed that chondroma of the testis exists only as a one-sided development of a teratoma in which other elements have been suppressed.

So that beginning with this extensive series we may now eliminate as separate tumors, myoma and chondroma.

A few cases of pure fibroma of the testis have been described in the literature, and are possibly genuine. They are so scarce, however, as to have no clinical significance.

As for myxoma, no genuine cases of pure myxoma of the testis have been described in the literature.

Lipoma is sometimes mentioned. There is no case of pure lipoma of the testicle on record, although this tumor is rather common in the spermatic cord, where, however, it is often associated with teratomatous elements.

While many of the adenocarcinomata are supposed to be derived from the seminiferous tubules, genuine pure adenoma of the testicle is almost never seen. Adenoma is practically limited to portions of the mixed tumors, and a pure simple adenoma of the testicle I have not been able to find, with one exception. Pick and Chevassu report cases of multiple adenomata in undescended testes. Three or four small nodules in the body of the testicle were found, looking like solid, well circumscribed, fatty areas. The tissue was made up of neoplastic seminiferous tubules; and it was believed to be the only true adenoma of the testicle. The conclusion, therefore, is that true adenoma of the testis arising in the seminiferous tubules exists, but is very rare and of no clinical importance.

In sarcoma one has to deal with a more complex group. Alveolar sarcoma figured largely in the diagnoses of the older surgeons, but Langhans showed that this tumor belongs with the common carcinomata. A famous group of sarcoma has been described, by French observers especially, under the term "sarcome angeioplastique." In recent years it has been shown that this tumor is identical with the growth aping the chorionic structures, and may properly be called a chorioma of the testicle.

There are a number of side lines of evidence which led to the recognition of these tumors as identical with chorioma. It has been shown that these choriomata are portions of teratomata and that they are often composed of all three germinal layers, so that chorioma as a simple tumor may also be thrown out.

Lymphadenoma has a lengthy history, especially in French literature. It is usually described as round celled sarcoma, often as a small round celled sarcoma. It was pointed out by Malassez as a highly malignant growth composed of small round cells, and called a specific type of sarcoma. The latest German writers also refer to this growth and they are somewhat undecided as to its exact significance. The most competent discussion of lymphadenoma is that by Chevassu (Thèse de Paris, 1908). He points out that in the reports of lymphadenoma of the testicle the size and shape of the cells vary, that the stroma is not always present, and that the clinical history does not always show differences from that of carcinoma of the testicle. He has himself examined several cases now standing in French literature as lymphadenoma and he has concluded that they are a form of carcinoma, and he states that the pathologists who made some of these diagnoses have stated to him that they have changed their opinions. He concludes that lymphadenoma as a primary tumor of the testicle does not exist. So that in the opinion of the most competent French observer this type of tumor is now eliminated from the group of primary neoplasms of the testicle. I have felt that many of the cases in the French literature were not genuine cases of lymphadenoma, and I was partly prepared to accept Chevassu's conclusions. Therefore, under the heading of sarcoma a third group may be struck out.

We now come to a little more difficult field, that is, the spindle celled sarcomata. There are about six or seven cases in the literature which one can not eliminate; some of them are old, some recent. The most satisfactory case is the one reported by Chevassu in 1907, which was of large bulk, and was composed chiefly of spindle cells with mucoid stroma and many giant cells. He studied all portions of the tumor, but failed to find

any other element. He would not state positively, however, that this was a pure spindle and giant celled sarcoma of the testicle, but said that it might perhaps be a mixed tumor. The later literature contains a number of cases of spindle celled sarcoma, but the descriptions do not read in a straightforward manner. There are a number of features about the descriptions which make me think that it would be wise to defer judgment as to the occurrence of this tumor in the testicle in pure form.

Hansemann described a tumor which he derived from the large interstitial cells of the testicle. His article was brief and the descriptions were incomplete, and it would seem that he intended more to suggest the possibility of the existence of such a tumor, rather than to make a positive claim. Not much attention has been paid to the interstitial cell tumors of Hansemann, but they have been classed among the sarcomata. The existence of such a tumor has not been demonstrated at the present time. The most recent contributions show that at times the interstitial cells multiply to such an extent as to produce a tumor-like process, but never reach the grade of a genuine neoplasm. Chevassu and others have discussed the part played by interstitial cells in sarcoma of the testicle and their conclusion is that there are no malignant tumors of this origin, that the forms of hyperplasia observed are more or less functional.

There remains then simply the old round celled sarcoma of the testicle, large or small, alveolar or diffuse. This is the commonest diagnosis made to-day on tumors of the testicle. There are different views in regard to the nature and origin of this tumor. Probably most writers at present believe that the round celled sarcoma is a carcinoma of the embryonal type derived from the cells of the seminiferous tubules. Chevassu thinks that he can demonstrate the origin from cells lining the seminiferous tubules. He admits that he could not trace the tumor directly to the lining cells of the tubules, but on general grounds he concludes that they are derivatives of these cells. He finds that the tumor cells exactly resemble certain cells of the tubular lining, but he lays stress upon the fact that this tumor develops within

the testis, supplants testicular tissue, and remains inclosed in the tunica albuginea for a long while. It would seem that Chevassu and others are assuming too much. The morphology of the cells of this tumor is not sufficient proof that they have developed from the lining cells. Moreover, one must remember Ribbert's opinion, that one can not tell from the examination of advanced tumors what relation the cells of origin bore to the surrounding tissues.

From the study of a series of cases I have reached the conclusion that all the common carcinomas and large round celled tumors of the testicle, including the "seminome" of Chevassu, are one-sided developments of teratomata. My opinion is based chiefly upon specimens of two cases which I have to show. One of these is a tumor of the testicle, which was received with the diagnosis of gumma, but which proved to be an interesting teratoma. This tumor consisted of two main portions: one a solid fibrous part, the other a soft medullary carcinomatous part. Several cross sections of a fibromuscular and glandular organ were found in the hard part. There were three lobules of tissue identical with normal adult human thyroid. I am aware that Aschoff has warned against fantastical interpretations of such findings, but I am confident that the fibromuscular organ in this teratoma was either a deformed uterus or possibly a stomach. I am, however, much inclined to consider it a uterus. Such an organ has not been previously observed in these tumors, nor has the presence of thyroid tissue been previously described. Here there are two adult organs, one possibly a uterus, the other certainly thyroid tissue, while the entire mass was surrounded by a diffuse carcinoma of a very characteristic type commonly seen in teratoma of the testicle. This carcinoma is characterized by the presence of large polyhedral cells supported by a stroma richly infiltrated by lymphocytes, the stroma appearing to be an integral part of the tumor. As to the explanation; one possibility is that we are dealing with two tumors, one giving rise to thyroid and uterus, and the other to the carcinoma or the seminome of Chevassu. The other possibility is that the tumor is a

teratoma of which the different elements have given rise to both portions of the tumor. The latter explanation seems to me to be preferable. In order to assume that the carcinoma was developed from the seminiferous tubule cells, one must suppose that he is dealing here with two distinct tumors.

The second original case on which I base my conclusions is a very early tumor of the testis in a physician, thirty-seven years of age. This tumor was about 1 x 2 cm. in size, and was composed of two portions separated by a mass of fibrous tissue. Portion 1 is typical chorioma. Portion 2 is typical large celled carcinoma, and in the center there is a small area of fibro-cartilage with a few canals lined by high cuboidal epithelial cells. There is no question that this is a tumor of Wilms' type, since it contains derivatives of all three germinal layers. The reason why cases showing this type of carcinoma have been separated from teratoma is that in them nothing but carcinoma has been found. Here is a very early case of carcinoma in which a small island of cartilage was found together with chorioma. It seems quite probable that if either one of these epithelial elements had been allowed to grow it would have suppressed the mesodermal structure, and would have been the only apparent element in the tumor. Fortunately this case was obtained very early, and thus its teratomatous nature was recognizable. I can not, therefore, accept Chevassu's conclusion that his carcinoma was derived from the seminiferous tubules, since I have found in one such case an island of cartilage indicating a teratomatous origin. This conclusion is offered as an hypothesis which can be worked upon, and I am gathering more material for study in this direction.

If this very complex group of tumors of the testicle can be simplified in this way, the question arises whether similar principles do not apply in other regions, and this possibility seems to lend increased importance to the study of testicular tumors.

The conclusions reached are briefly as follows:

The tumors occurring in the testicle are: fibroma, spindle

celled sarcoma, multiple adenoma, and teratoma with its derivatives.

Pure fibroma, spindle celled sarcoma, and adenoma are very rare; teratoma is common.

Myxoma, myoma, chondroma, carcinoma, and large round celled sarcoma or carcinoma occur in the testicle only as one-sided developments of teratomata.

Discussion.

Dr. W. G. MacCallum said that although he had been attacked by everyone who had written on testicular tumors since the publication of his own case, he had not taken further occasion to refer to it. Dr. Ewing had gone through the whole list of tumors of the testicle, and had ruled out one tumor after another as being after all of teratomatous nature. It seemed that a line of division might be drawn between teratomatous tumors as such and those in which one element of the teratoma, instead of remaining merely distorted tissue, had taken on the peculiar characters which are distinctive of invasive malignant tumor growths. In this sense it was very difficult to say that any malignant tumor was not primarily of teratomatous origin. In the case to which he had referred, the tumor consisted apparently of one type of tissue in addition to the loose myxomatous stroma. It was typically a malignant tumor growing with extreme rapidity into the veins and extending widely throughout a great part of the venous circulation, even filling up the jugular veins and some of the cranial sinuses. Although it was perfectly possible, and perhaps probable, that this tumor arose primarily from misplaced embryonic structures in the testicle, there was nevertheless the typical alteration in these tissues which gave it its malignant character. In view of this fact, it seemed hardly more necessary to speak of this tumor as a teratoma than to apply the term to most other malignant growths. The conditions would have been very different had other types of tissue been found in the primary growth. Dr. MacCallum thought that the group of

tumors mentioned by Hansemann offered a field of especial interest in the determination of their origin, for it would be necessary, since they seemed allied to the interstitial cells, to determine whether or not they were mere hyperplasias of these cells before classing them with sarcomata or other tumor subdivisions.

REGENERATION OF LIVER TISSUE.

LINDSAY S. MILNE, M.D.

This subject, which has undergone such varied and extensive discussion, may frequently be well illustrated in many diseases of the liver. One of the best examples of the phenomena of compensatory reformation of liver tissue occurs in the disease known as acute liver atrophy, and is especially evident in its more subacute types.

Cases of acute liver atrophy vary greatly in their clinical histories and also, in more or less corresponding degree, in their pathological appearances. In the cases in which the patient succumbs early in the disease, the essential histological feature is a widespread necrosis of liver tissue. In slightly more protracted types, the destroyed liver cells begin to disintegrate and disappear, and the capillaries between which they lay become in corresponding degree dilated. The skeleton of the lobule, in consequence of this liver cell destruction, tends, to some extent, to collapse and, in accordance with the duration of the disease, a new formation of connective tissue spreads into the destroyed lobule from the region of the portal spaces. As the duration of the illness increases, owing possibly to a better resistance of the patient or to a less virulent type of the disease with less extensive liver destruction, the necrotic tissue becomes more completely replaced by fibrous tissue, until eventually, in the late types, only islets of liver cells are included among extensive areas of fibrous tissue.

In the subacute stages of the disease, the remaining undestroyed liver cells can be observed to have assumed a very active hyperplasia. To illustrate some of the different stages and appearances of this subacute type of liver atrophy, I may, in brief, present three cases which occurred in children. At this time of life the subacute variety seems to be relatively the prevalent type of the disease.

Case 1.—Occurred in a well nourished boy, six years old, who had died after an attack of gradually deepening jaundice of eight weeks' duration, with no other particular symptoms having been observed, save those of apparent heart failure toward the end. Even at the time of death the jaundice had not assumed any very marked degree. At the autopsy, the liver was slightly reduced in size. As a whole, it was dark red in color, but on its surface were several large, irregular, slightly elevated, bright yellow patches. On section of the organ, these yellow areas were rather ill-defined and were irregularly arranged among a red, firm tissue. The bile passages showed no particular abnormality.

Microscopically, the yellow areas proved to be liver cells which had apparently escaped destruction, and although some were somewhat degenerated, they were for the most part in an actively hyperplastic state. The lobules were larger than normal and more irregular in outline, while the individual liver cells forming the trabeculæ had apparently also become very irregular in size and shape, some being large and pale from hypertrophy, others small, darkly staining both in cytoplasm and nucleus and arranged in clumps.

There were also numerous giant cells, each containing several nuclei. Occasionally karyokinetic figures were seen in the liver cells, but evidences of amitotic cell division were much more common. Apparently every stage of this latter process could be observed, independent of any phase of karyokinesis. This process consisted apparently of a swelling of the nucleus and cytoplasm followed by a condensation of the chromatin, fission of the nucleus and a subsequent cleavage of the cytoplasm.

In this process of rapid amitotic liver cell division multinucleated giant cells appeared to have been formed frequently, and there were some evidences that they also sometimes seemed to have split into clumps of small, darkly staining liver cells.

The intervening red substance between the yellow areas was composed of skeleton lobules made up of distended capillaries from which the liver cells had been completely removed. The lobules were somewhat collapsed, and a new growth of young, cellular fibrous tissue from the portal spaces had replaced about half or more of the outer part.

Case 2.—Occurred in a very well nourished girl, four years old, who had succumbed after a forty-day history of progressive jaundice with practically no other special coexisting symptoms. Twenty months previously she had suffered from a somewhat mild attack of jaundice of two months' duration. She died of gradual heart failure and without any supervening cerebral symptoms. The autopsy revealed a liver somewhat less in size than normal, extremely firm, and of a dark red color. Projecting on the surface, particularly near the portal fissure, were a considerable number of large, rounded, bright green colored, tumor-like masses of varying size up to that of a walnut. In the interior of the liver also there were numerous large, well defined, green areas.

Microscopically, the green nodules were found to be composed of liver cells, extremely irregularly arranged, partly degenerated, but showing numerous signs of recent and also of old hyperplasia. Many of the liver cells were large, irregular and evidently hypertrophic, and large numbers had assumed the appearances of giant cells; as many as thirty nuclei being present in some of them. Nuclear division by amitosis and also rather frequent karyokinetic figures could be observed. The intervening fibrous tissue was rather dense and had completely obliterated all lobular structures. Possibly in this case the initial destruction had taken place two years previously, coincident with the first attack of jaundice. Some more recent toxic change had destroyed the balance of liver tissue and so led up

to the terminal issue, and had also been responsible for the evident recent signs of degeneration and of recent liver cell hyperplasia.

A common type is also illustrated in a well developed girl six years and eleven months old, who presented a history of an attack of jaundice a year previously, since when she had occasionally complained of diffuse pains over the epigastrium. For the last seven weeks she had been looking sick and the development of ascites had been noticed. During the last five days of life she had been slightly jaundiced and she had been getting increasingly torpid. She died after having been comatose for a day, during which time she had also frequent convulsive movements of the face and extremities. At the autopsy a large amount of ascitic fluid was found in the abdominal cavity. The liver was extremely small, being of a pinkish-gray color, and from its surface there projected numerous, irregular, fairly large, well defined yellow nodules. These nodules were composed of masses of closely packed liver cells having no trabecular arrangement and individually being very irregular in size and shape. The intervening tracts of fibrous tissue were very dense and contained only a few rather attenuated "ducts." Both macroscopically and histologically the appearances in this case were too uniform to simulate an ordinary coarse cirrhosis, which naturally is very irregular, owing to its being due to a long continued, frequently repeated damage to small areas of liver tissue and not to a widespread, rapidly accomplished destruction as in acute and subacute liver atrophy.

In ordinary atrophic cirrhosis of the liver, the extremely complicated picture which is seen of collections of irregularly arranged and variously shaped liver cells included in tracts of fibrous tissue is not altogether due to a simple replacement of areas of necrotic liver substance by fibrous tissue, but compensatory liver cell hyperplasia is also to a large extent accountable.

Besides the rapidly accomplished destruction of liver tissue which occurs in acute liver atrophy, and that which occurs more slowly giving rise to common cirrhosis, all other extensive dam-

ages to the liver are apt to be associated with evidences of liver cell hyperplasia, being especially marked when the volume of the liver is seriously reduced somewhat rapidly. In a case of leukemia, I have seen large numbers of liver cells in karyokinetic division as well as very frequent evidences of ordinary amitosis. Around lymphadenoma nodules in the liver in one case there were enormous numbers of liver cells in all phases of mitosis. The liver cells, in this case, around the nodules were in an extremely active state of hyperplasia. Almost similar appearances may often be seen around multiple malignant tumors in the liver. In such conditions as chronic venous congestion, where a great deal of liver tissue has become lost by atrophy, there are commonly apt to occur extensive signs of liver cell multiplication and particularly hypertrophy. In conditions, then, demanding compensatory increase in function of the liver cells, there is apt to be both a hypertrophy, and also a multiplication of liver cells directly and with no transition in type through "bile ducts" or other means. The main method of cell division is by amitosis, although mitosis in the early generations of liver cell new formation can fairly commonly be observed.

Experimentally, compensatory liver cell regeneration can also easily be demonstrated. This regeneration depends largely for rapidity and for extent of development on the amount of liver tissue removed. Thus in the rabbit, three weeks after removal of half the liver, the remaining half, throughout, shows very marked evidences of regeneration. The lobules are very much larger than normal, some of the individual liver cells, particularly those towards the periphery of the lobules, being very hypertrophic, while others, mostly near the intermediate zone, seem to have split into a large number of small, darkly staining cells. Mitosis in this new development, again, is only rather rarely seen, and, I think, occurs to a marked extent only in the early stages. Amitosis, as also apparently in human conditions, seems to be the method by which the main part of the new liver cell multiplication is accomplished. In twenty-four to forty-eight hours after removal of half the liver in rats and rabbits,

the liver cells at the periphery of the lobules are evidently enlarged, pale and hypertrophic. From the fourth to the eighth day evidences of new formation of liver cells can generally be distinguished, and during this period karyomitotic figures may fairly frequently be observed. In two to four weeks the multiplication of new liver cells is at its maximum.

Another method of liver cell regeneration is commonly accredited to the small, "bile-duct structures" which are frequently noticed ramifying amongst the fibrous tissue in numerous pathological conditions of the liver. These have very commonly been considered as at least an attempt at new formation of liver cells. These "ducts" are seen well in all recent developments of fibrous tissue, as in subacute liver atrophy and in certain of the more acute types of cirrhosis, while in chronic dense fibrous formations they are either very fine or cannot be observed at all. In the well-defined strands of fibrous tissue in old quiescent atrophic cirrhosis, for example, there are often none of these "ducts" to be observed. In acute liver atrophy, and also in all kinds of recent inflammatory change in the liver, they can often be seen in direct communication with old interlobular bile ducts. Their lining epithelium is generally very regular in type, but sometimes shows catarrhal changes and may form small, club shaped masses of cells. The neck where the new structure joins the interlobular bile duct is usually rather fine, or lined by a very regular type of epithelium, and I have never seen any definite evidence that these ducts are derived by a proliferation or sprouting from an interlobular bile duct: Undoubtedly, catarrhal proliferative changes do often occur in these interlobular bile ducts, as one would expect from the action of the irritants, necessarily associated with most pathological conditions of the liver, being eliminated down them. Artificially also catarrhal changes may be produced with marked multiplication of the lining cells by the injection of pancreatin, etc., into the bile ducts, or as the result of general toxic administrations. In these proliferative states, no signs of sprouting out of duct-like structures can be observed. Serafini has also shown the remarkable rapidity and

completeness of regeneration of the epithelium of the bile passages. As a result of his experiments, it appears that in eight days after the lining membrane of the gall bladder has been scraped off, a new epithelium had completely reformed.

These "ducts" have also been considered to be derived from atrophied liver cells, but in conditions of atrophy from chronic venous congestion, in waxy disease, in congenital syphilis, etc., the atrophying liver cells do not really closely resemble the fresh-looking, regularly lined "ducts" familiarly seen in fibrous tissue developments in the liver. Experimentally, also, transplanted pieces of liver of the same animal do not show in their regressive changes any condition resembling the "ducts." Out of a series, in only two rats did the implanted liver cells persist for any considerable time. In these the liver cells could still be recognized after three months. They had not proliferated at all and were very considerably split up by inflammatory cells.

In certain cases of liver cirrhosis these apparently new "ducts" are found in great numbers, and by serial sections they can frequently be traced into interlobular bile ducts and also directly to trabeculæ of liver cells, and they generally join either of these structures more or less abruptly, with no transition in type of lining cell. A good case illustrating these connections was observed in the liver of a child four months old, who had died after an attack of jaundice which had lasted since the day after birth, and was due to a congenital obliteration of the bile ducts. In this case the liver was greatly enlarged and intensely bile stained. The peripheral part of each lobule was occupied by a zone of young fibrous tissue, in which ramified enormous numbers of bile-duct structures. These "ducts" with great frequency communicated with the interlobular bile ducts, easily distinguishable from them, however, as they were lined by a somewhat different type of epithelium, and as the latter frequently possessed a very delicate elastic lamina. They also, with even greater frequency, communicated with liver-cell trabeculæ having a lumen perfectly continuous with the intratrabecular bile collecting canaliculus. Even in one high-power field of the mi-

croscope, these "ducts" could sometimes be seen joined to both an interlobular bile duct and to a trabecula of liver cells, there therefore being a complete excretory channel from the liver cells. Their epithelium was very regular in type, except sometimes just at the communication with liver cells or bile ducts, at which places it occasionally tended to appear fine, rather like a capillary. The extremely tortuous and ramifying character of the "ducts" in this case, as also in other pathological conditions of the liver of children, may to a large extent be explained by the well-known irregular and branching course of the connecting bile canaliculi in the small livers of early childhood as compared with the more direct character prevailing in the fully developed adult liver.

It might seem then as if these "ducts" were simply the becoming more evident of normal bile canaliculi, isolated from their normal envelopment of liver cells, and in their new environment having assumed this larger type of lining epithelium. Similar processes to this can also be seen in the flattened lining cells of the air alveoli of the lung in conditions of interstitial pneumonia, in Bowman's capsule of the glomeruli in various kidney diseases, etc.

I might also briefly mention another case of subacute, or what might be called chronic, liver atrophy which appeared to be somewhat illustrative of these processes. It occurred in a fairly well-nourished man, aged forty-one years, who had died after an eight-months' history of mild jaundice, which had, however, begun rather acutely. He had no particular gastrointestinal symptoms, with the exception of hematemesis, during the last week of life. There was, however, a history of nephritis and cardiac failure for fifteen months, and for the last five months there had been a gradually increasing ascites. He died of purulent peritonitis. The liver, at the autopsy, was found to be enormously reduced in size, weighing only 700 grams and measuring only 6 cm. in thickness in its central part. Its surface was smooth, as a whole, but especially on the upper part of the right lobe and near the hilum numerous brownish-green no-

dules of varying size projected. The interior of the liver was mainly made up of pink-colored, firm fibrous-like tissue and embedded in this were scattered, irregular, brownish-green masses. Microscopically, these latter areas were composed of closely packed hyperplastic liver cells. The intervening fibrous tissue was dense and contained relatively few "bile ducts." In some few parts, however, there were considerable numbers of rather attenuated "ducts." Here some recent toxic process seemed to have destroyed the liver cells which previously occupied this area. The trabecular structure and approximately normal capillary arrangement could still be made out and the intratrabecular bile canaliculi which had in large part escaped this destruction appeared in some places as fine, undifferentiated tubes, sometimes being slightly dilated; in other places they had a lining of very flattened endothelial-like cells, and in still other places the epithelium had assumed a cubical definitely "duct" appearance. There were then in these areas apparently all stages in development of definitely cubical lined "ducts."

In cirrhosis of the liver the apparently new "ducts" are commonly seen either to end in a clump of definite liver cells, or else to have fully formed liver cells included in some part of their course. At first sight it would seem as if the "ducts" were forming the liver cells, or possibly that the liver cells were originating the "ducts," or even that the "ducts" might have some purposive quality of growth attempting to connect liver cells with interlobular bile ducts. It seems easier, however, to understand, in the absence of proliferative evidences, that the liver cells thus included in, or in relation to the end of, the "duct" are merely the old pre-existing liver cells and that the "duct" has become more evident simply as a result of a change in type of the epithelium lining the collecting canaliculus, which has resisted the destruction responsible for the removal of its enveloping liver cells.

Experimentally, also, the function and fate of these "ducts" may be studied, in wounds made in the liver. Their appearance does not seem to be dependent on the amount of damage done

to the liver, as is seen to be the case in liver cell multiplication. They may be seen frequently at the edge of the wound in four or five days, often in connection with exposed ends of liver cell trabeculæ which do not seem to show any definite evidences of proliferating them. They may also be seen in the neighborhood of portal spaces, involved in the edge of the necrosis and to be carried irregularly outward from these positions by the development of new inflammatory tissue. At first they are always fine and somewhat like a capillary, but soon their epithelial lining cells swell up and the familiar "duct" is to be seen. In three or four weeks these "ducts" are usually most definite. They get progressively more distorted from their normal positions by the development of inflammatory tissue, but after six weeks they begin to show signs of atrophy. In two or three months they are usually very atrophic-looking, and often no more can be seen than in the fibrous tissue strands of a quiescent type of cirrhosis. They seem then to be derived from a swelling up of the lining epithelium of the normal canaliculi leading from the liver cells to the interlobular bile ducts. These structures are naturally more resistant to destructive influences than are the liver cells and, persisting in their new inflammatory environment, assume the well-known regular "duct" type. Their lining epithelial cells may occasionally show proliferative changes, but these are due to catarrhal changes induced by irritants in process of elimination inside the "duct," or to the proliferative changes which might be expected in this epithelium disturbed in its normal function and involved in a new environment.

Regeneration of liver cells takes place very freely, following any rapid extensive reduction of liver tissue and is accomplished by a multiplication of pre-existing liver cells, chiefly by amitotic division, the early phases apparently also being initiated by the karyokinetic method.

Discussion.

Dr. Richard M. Pearce said that he had been particularly impressed with the great resemblance between this lesion and that which he had described some time ago in the dog, caused by the use of hemagglutinative sera. In the first place, the acute lesion produced experimentally was microscopically very much of this type. There, also, large areas of liver were destroyed. In the second place, the mitosis of liver cells took place at about the same distance from the necrosis; that is, in about the third or fourth row of cells from the area of destruction. The giant cell formation occurred to a wonderful extent in these animals, and could be explained only by an atypical type of proliferation of liver cell nuclei about necrotic cell fragments. What their fate was he did not know. He gathered from what Dr. Milne had said, that in this human lesion the cells split up into smaller cells, and he would be very glad if Dr. Milne could say something more about the fate of these cells: whether there was a further cell division or not into fairly typical liver cells. In regard to the bile-duct question, Dr. Pearce said that although he considered the formation of liver cells from the newly formed bile ducts to be the more common condition, in some of his lesions he had felt that there could be traced a transformation from true liver cells into these so-called false ducts. In the experimental lesion a very large portion of these newly formed ducts were essentially solid cell processes, and as such he did not see how they could be interpreted as the remains of pre-existing ducts; they must be considered as the result of proliferation of pre-existing ducts and analogous to the angioblasts of granulation tissue.

Dr. W. G. MacCallum thought that studies of such cases as were referred to by Dr. Milne showed pretty clearly two methods of regeneration of liver tissue: one in which the remaining liver cells gave rise by mitosis to new liver cells; and the other a less perfect method and one usually cut short by the death of the patient, a new formation of liver cells by the

sprouting of the bile ducts. The mitotic division of the liver cells appeared almost simultaneously very soon after the primary injury, while the proliferation of the bile ducts seemed to go on much more slowly. The structures commonly spoken of as newly formed bile ducts in cases of regeneration after acute yellow atrophy and in cases of cirrhosis of the liver seemed to him to be really newly formed bile ducts, at least in part; while in part they were the remains of the more resistant original ducts. In their growth they might fulfil two ends, either to re-establish connection with the newly formed liver cells, or to form at their budding ends new liver cells. In one or two cases of very advanced cirrhosis of the liver it had been possible to demonstrate this to his satisfaction, inasmuch as the cells of the liver tissue in general were deeply pigmented while the bulbous masses of new liver cells on the ends of the sprouting bile ducts were evidently fresh and stained pink without any pigmentation. In some cases mitotic figures could be observed in these bulbous ends. This was particularly evident in one case in which after extensive destruction of the liver cells the skeletons of the lobules remained intact, and into the site of the old liver cells such bile ducts could be seen sprouting radially from all sides of the lobule.

Dr. Pearce said that he did not wish to be understood as stating that the formation of bile ducts from liver cells was a common process; on the contrary, he considered it a most unusual process.

Dr. Harlow Brooks said that he had been extremely interested in this presentation, but he had disagreed with some of the conclusions. Dr. Milne had stated that the clinical picture of these cases was identical with cirrhosis. Dr. Brooks' experience had been confined to three cases, only one of which had come to autopsy. This was absolutely not the same condition. He believed that in the next few years, after more careful study, it would be possible to make a sharp differentiation, on the one hand from acute atrophy of the liver, and on the other from cirrhotic conditions. In the first place, in cirrhosis the vascular

changes and symptoms are pronounced. In these cases, so far as he had seen, the vascular symptoms do not occur. On the other hand, they were sharply differentiated in the lack of toxic conditions so manifest in cases of acute atrophy and infective icterus. These cases were strikingly free from symptoms of poisoning which one associates with practically all acute degenerative processes in the liver. There was also an absence of fever and an absence of the urinary findings which one gets in acute atrophic conditions. He thought that this was a distinct disease which could be recognized by its clinical symptoms.

Dr. James Ewing said that he was much interested in the clinical aspect of these cases. He had always felt that the scope of the term acute yellow atrophy of the liver was very much wider than the textbooks indicated. There were a large number of cases occurring in subjects who recover, and he thought that it would be a distinct advance when the particular clinical conditions which lead to acute yellow atrophy of the liver followed by recovery were recognized by the clinicians. Dr. Ewing had seen conditions clinically in which he felt that a very considerable destruction of liver had occurred, and yet the patient recovered. It seemed to him that the clinical side of this study was quite as important as the study of the minute anatomical changes.

Dr. Brooks said that the histories of these cases had been studied very carefully. The patient referred to was an intelligent man who was in the wards for four months, and who absolutely denied any such acute attack, as it was thought he must have had. On the other hand, he never showed any signs of getting well; he grew steadily worse.

Dr. Horst Oertel said with regard to the relationship of bile ducts to liver cells, that, while his study of the experimental lesions was limited, in a considerable study of human lesions he had never seen any pictures which indicated to him a transformation of bile ducts into liver cells. He had at times seen specimens which might impress one as a transformation of liver cells into bile duct epithelium; but of late he had become doubtful

about that transformation. He had formerly been accustomed to speak very liberally of bile duct proliferation; but he had been much impressed with Dr. Milne's study and it appealed to him particularly because it had considerable analogy to parenchymatous changes in other productive inflammations. In the productive and tuberculous inflammations of the lungs, for instance, no one would think that the transformation of alveoli into glandular loops represented newly formed structures, but old alveolar epithelium, which under changed environment had changed its form. Similarly in the productive nephritis, we regard the new adenomatous loops, so frequently found, not as new productions, but old tubules and cells which, under changed surroundings, have changed their type. One hesitates to accept, still, the old idea of so-called excessive bile duct proliferation in productive hepatitis and the transformation of liver cells into bile ducts. Without absolutely denying the latter, Dr. Oertel felt quite strongly that this was not a general process.

Dr. Milne said that the early cases which he had described showed nothing more in the clinical histories than a gradual development of jaundice. The child whose illness had lasted six weeks felt perfectly well up to within a few days before death. There was no other condition than jaundice; no pain or other symptoms. In regard to the giant cells, Dr. Milne said that there were some evidences that they split into a number of small cells.

A PRACTICAL IMPROVEMENT IN THE PREPARATION OF ANILIN-GENTIAN-VIOLET STAIN.*

BY JULIUS SHARNOSKY.

(Laboratory Helper, Bellevue Hospital.)

Paltauf's modification of the anilin-gentian-violet solution was called to my attention by Prof. William Elser. I have been unable to find any reference to a published description of the

*Read in abstract by Dr. Charles Norris.

method, but learn that it has been in use for many years in Vienna.

The advantages of this staining fluid consist in the uniformity of its preparation, its permanence, and the absence of precipitate upon the slide. The formula for its preparation, and the time limits for the staining with this solution and with Gram's iodine solution and absolute alcohol (for all of which I am indebted to Dr. Elser) are as follows:

3-5 cc. Anilin, Merck's.
90 cc. Distilled water.
7 cc. Absolute alcohol.

Shake thoroughly; filter through moistened filter paper, and make sure that solution is clear. Then add

2 g. Gruebler's powdered Gentian Violet.

Allow fluid to stand for twenty-four hours, on account of precipitation which takes place. Filter before using.

The solution is good only when a metallic lustre develops on the surface of the fluid. This solution is said to keep from four to six weeks in a stoppered bottle which has been thoroughly cleaned.

Application:

Anilin-water gentian violet..... 3 minutes.
Gram's iodine sol..... 2 minutes.
Absolute alcohol, with stirring.....30 seconds.
Weak carbol-fuchsin, or aqueous fuchsin..30 seconds.

The times stated above should be strictly adhered to, and the preparation should not be washed in water until the counter staining is finished.

The great importance of the anilin-gentian-violet stains in routine bacteriological work has led to a series of experiments with a view to improving them, especially with regard to permanency. The difficulty in preparing Gram's stain is well known. The disadvantage consists in not always obtaining a clear solution of anilin oil in water, the stain, when finally pre-

pared, keeping at most two weeks, at the end of which period it begins to lake, complete deterioration taking place. In large laboratories the annoyance of continually having to prepare fresh stains, and the frequent necessity of waiting till a fresh product can be prepared, made the desirability of conducting experiments with the first stated view in mind most keenly felt.

As a result, a stain has been produced embodying both stability and ease in preparation. This was accomplished through the use of a salt of anilin, instead of the base. This salt is a white crystalline body, much more soluble in water and in alcohol, yielding a clear solution, and possessing, at the same time, a higher grade of stability. The formula, as finally developed, is given below, together with a comparative table of the different anilin-gentian-violet stains in use.

Formula:—Dissolve 3.5 g. of anilin hydrochloride (Kahlbaum's) in 90 cc. of water; add 10 cc. of absolute alcohol; warm to about 60° C. and add 1 g. gentian violet slowly, with constant stirring; digest over a small flame for about 10 minutes and filter.

It will be seen that the characteristic metallic luster develops at once, thus doing away with the necessity of waiting twenty-four hours before the stain is ready for use. The product prepared as above has kept ten months to date, without the slightest signs of deterioration.

Perhaps it would be advisable to say a word regarding the addition of 10 cc. of alcohol. In the old formulas, alcohol, of course, serves to facilitate the solution of anilin. But one may very properly question the necessity of using alcohol in our formula, in view of the solubility of the anilin salt in water.

If we start out with the premise that the anilin hydrochloride, when purchased, is absolutely neutral, there is no reason for the addition of alcohol. However, the commercial salt invariably has an acid reaction due to hydrolysis, rendering the addition of a few drops of anilin necessary to effect a neutral

reaction,[1] and it is thus apparent that the use of alcohol is desirable.

	Anilin.	Alcohol.	Stain.
Ehrlich's Anilin Gentian Violet[2]	2.5%-3%	15%	0.5%-1%
Ehrlich's Anilin Methyl Violet[2]	2.7%	8%	1%
Stirling's Anilin Gentian Violet[3]	2.3%	10%	1%
Paltauf's Anilin Gentian Violet[4]	3%-5%	7%	1%
Bellevue Pathological Laboratory:			
	Anilin Hydrochloride.		
(Anilin Gentian Violet or Anilin Methyl Violet)	3.5%	10%	1%
	(Equivalent to 2.5% Anilin.)		

STRIATED CELLS IN THE FETAL THYMUS.

A. M. PAPPENHEIMER, M.D.

Dr. A. M. Pappenheimer demonstrated to the Society a microscopic section of the thymus of a five and one-half months human fetus, showing curious striated cells. So far as he knew, similar cells had never been described in the human thymus. They had the appearance of atypical striated muscle cells, such as are found in the teratomata and congenital rhabdomyomata of the heart. They were spindle shaped structures, most of them long, varying in thickness. The nuclei were oval, large, and situated on the surface. They all showed perfectly distinct cross and longitudinal striations. They were scattered through the lobules, usually occurring in groups, and showing a tendency to lie at the periphery. Such cells were first described by Mayer in 1888, and since then had been found in the thymus of various fishes, amphibians, reptiles, and birds. It had been spe-

[1]The term "neutral reaction" in this case is used from the standpoint of congo-red and fuchsin, and not as regards litmus or methyl orange.

[2]Either gentian violet or methyl violet may be used in either of these formulas, although methyl violet, on account of its greater purity, is to be preferred.

[3]Formula calls for 5g., but 1% (1g.) actually dissolves.

[4]Formula calls for 2g., but 1% (1g.) actually dissolves.

cifically denied that they ever occurred in the mammalian thymus. Their significance has been a matter of dispute. The majority of authors explain them as fetal inclusions. Other writers have considered them peculiarly specialized cells derived from the reticular epithelium of the thymus. These cells, according to Hammar, show a pretty definite fibrillar connection with reticular cells. Dr. Pappenheimer was inclined to agree with Hammar's view. One reason was that in this same thymus he had found rudimentary Hassall's bodies which consisted of two or three cells, some of the individual fibrillæ showing distinct cross striations. In other places, there was a connection between these fibrillæ and the reticular cells. The term "myoid cells" had been suggested for these structures.

A detailed description of these cells, with literature, will appear in the *Journal of Medical Research.*

DOUBLE HYPERNEPHROMA.

B. C. CROWELL, M.D.

Dr. B. C. Crowell showed a large tumor which had been removed at autopsy from a patient at Bellevue Hospital. The patient was a young girl, eighteen years of age, who had been admitted to the hospital in a practically moribund condition, so that but little history could be obtained. She said, however, that she had never been strong, though she had had no illness except typhoid fever. She had always been small. On admission she was found to be very much emaciated, the loss of weight having taken place within the last four months. During that time she had noticed an enlargement of the abdomen. She thought that at times her urine had been cloudy and bloody, and she had had some pain on urination.

At autopsy she was found to be much emaciated, with this very palpable tumor which lay on the left side practically dis-

placing everything, the kidney being pushed downward. The growth did not originate in the kidney itself, which was apparently intact except for slight metastases in the lower pole. The tumor had undergone extensive necrosis, and aside from the general appearance there was a considerable amount of fluid which looked very purulent, as though infection had taken place. Section of the anterior part showed a tumor juice exuding from it on pressure. A similar smaller tumor occupied the site of the right adrenal, displacing the kidney tissue downward. The ureters and bladder were perfectly free; there was no ascending infection. The kidneys showed minute abscesses, and abscesses were also found in the intestine and spleen. Metastases were present in the kidneys, in the retroperitoneal lymph nodes, and in the pleural viscera up to 3 cm. in diameter. Some very small nodules were found in the substance of the left lung. Section of the vessels was very unsatisfactory on account of the size of the tumor, so that whether or not there was tumor tissue in the renal veins or vena cava could not be determined.

The tumor was presented as a hypernephroma which was interesting on account of its large size, the two masses together with the kidneys weighing thirteen pounds.

A CASE OF PRIMARY TUMOR OF THE VELUM WITH MARKED COMPRESSION OF THE FOURTH VENTRICLE.

HARLOW BROOKS, M.D.

The patient was a girl aged eleven years. She first came under observation of Dr. J. Constantine MacGuire, to whom I am indebted for the history and most of the clinical notes of the case. The child was born of nervous and excitable parents and from early life had been the witness of constant domestic disturbances, so that the nervous condition that she presented had

been looked upon as fully accounted for. She had suffered from various diseases of infancy, and had always been considered frail, although of about normal size and development for her age. Previous to coming under the care of Dr. MacGuire, she had been ill for two weeks, suffering from a condition diagnosed as "brain fever."

On examination she showed general muscular rigidity of a spasmodic character with slight opisthotonos. She rolled and tossed about the bed, was illogical, and had at times to be restrained. Occasionally she screamed and cried hysterically. She complained rather persistently of pains in the head. The knee jerks were accentuated; there were no ocular disturbances manifest; and examination of the eye grounds was said to be negative. There was no Babinski reflex; and no elevation of the temperature; and the pulse varied from rapid to slow. At this time a diagnosis of probable meningitis was made.

The child was removed from the surroundings which were manifestly bad for her condition and was placed under observation in a hospital. Here she was actively purged and put upon vigorous doses of the bromides. Under this treatment the symptoms apparently entirely cleared up, the muscular rigidity passed away and the child was soon able to sit up and to walk about the ward. Mentally the condition became apparently normal, cerebration and speech were quick and apparently natural in every way and the patient was cheerful and uncomplaining. Medicinal treatment was then entirely suspended, but the child remained under observation. With this startling result following treatment naturally a diagnosis of hysteria was made.

Some days after apparent recovery, a parent of the child was permitted to see her and, unknown to the attending physician, brought a detective who sharply questioned the child as to alleged misdoings on the part of the other parent. The girl became very much distressed and excited, and shortly after this ordeal developed convulsive attacks of the same nature as those previously present, during which she soon died.

Post-mortem examination shortly after death showed a

practically normal condition throughout the entire body except for the following lesions in the brain which are quoted verbatim from the protocol.

The skull, scalp, and dura mater are entirely normal, but the blood vessels of the pia arachnoid are moderately injected. The markings of the brain are regular, symmetrical and typical. The convolutions are, however, remarkably flattened, and palpation of the hemispheres shows fluctuation. On removal of the brain from the skull the infundibulum was found to be greatly distended and on its rupture about 500 cc. of clear cerebrospinal fluid escaped. The sella turcica is somewhat excavated as a result of the infundibular distention, but the pituitary body is apparently normal. The optic chiasm is compressed by the bulging infundibulum, and its bands are thinned and slightly atrophied.

The foramen of Munro is greatly dilated, and on opening the lateral ventricles they are found to be enormously distended with cerebrospinal fluid. The choroid plexuses are anemic, but otherwise apparently normal.

Section through the cortical tissue of the cerebrum shows marked anemia but otherwise, aside from compression, it is apparently normal. The cortical gray matter is normally thick and regular.

Section of the pons, medulla, and cerebellum shows a large tumor mass apparently originating from the velum which covers in the floor of the fourth ventricle. As a result the floor of the ventricle and superjacent substance of the cerebellum are greatly compressed, but neither tissue appears to be integrally invaded.

On careful dissection the tumor can be readily separated from the cerebellum, but not from the floor of the fourth ventricle where it is firmly attached by a more or less clearly defined adhesive pedicle which covers in almost the entire ventricular area. The tumor is flattened oval in form; it measures six cm. in diameter, and three cm. from above downward. It is light

pink in color, very soft, and almost pultaceous in consistence; but it shows no areas of gross necrosis.

The superjacent cerebullum is very much compressed, but no areas of invasion or of degeneration can be made out in it. No strands of degeneration are grossly demonstrable in the tracts of the lower medulla or in the cervical cord, although the ventricle of this portion of the cord is somewhat dilated. The tumor has completely obliterated by compression, the iter and the passage below connecting the medullary ventricle with that of the cord; this is doubtless the explanation of the condition of hydrocephalus.

Microscopic examination of the tumor shows it to be composed of a diffuse mass of embryonic connective tissue cells which are for the greater part of the small round cell type. The stroma is very scanty and is largely composed of spindle-shaped cells which are evidently closely related to those which make up the chief mass of the tumor. Blood vessels are infrequent, but the tumor is rich in what are apparently lymph passages. The smaller channels are composed of irregularly arranged tumor cells, and only the larger vessels show a definite structure into which adult connective tissue and endothelium enter.

Portions of the velum are still intact in the growth, though the neoplasm has evidently sprung from the connective tissues of this structure. No direct evidence of invasion of the tissue of the cerebellum or medulla can be made out.

Histologically, I am inclined to class this tumor as a sarcoma of the small round cell variety, but in some respects it resembles a glioma. Taking into consideration, however, its evident point of origin, I believe that I am fully justified in the assumption that the growth is a sarcoma. From a clinical standpoint the case is chiefly interesting and remarkable in that with so marked a growth complete relief of symptoms took place from time to time. It is, of course, absurd to assume that the bromide had directly to do with this amelioration, but I am rather inclined to the idea that the catharsis with the mental quiet and rest which the child enjoyed after removal from her home

were partly responsible for the cessation of symptoms. It is, however, possible that the inhibiting action of the bromide on the activity of the ganglion cells really may have had a share in causing this improvement.

Manifestly, the convulsive and irritative symptoms manifested by the patient when she was first seen were due to the acute hydrocephalus. It is probable that with the rest in bed and quiet of the hospital a certain amount of absorption of this fluid with relief of the cortical pressure took place, as a result of which the temporary improvement noted occurred. It also seems probable that the excitement induced by the painful questioning of the sensitive child caused a cerebral hyperemia, with perhaps increased secretion of the cerebrospinal fluid, increased cortical tension and resulting convulsions and death.

It is extremely difficult to understand why choked disk, Babinski reflex, and other evidences of cerebral compression were absent in this case. The absence of disturbances of equilibrium with such marked cerebellar compression is truly remarkable, but no more so than the absence of respiratory and cardiac disturbances when the floor of the fourth ventricle was so universally and remarkably compressed.

In so far as I have been able to determine. the case is unique, not only in symptomatology, but also in the location and character of the tumor. As a whole, it only verifies the quaint statement said to have been made by Charcot: "There is no lesion of the central nervous system which may not exist at times without symptoms and there are no symptoms which may not appear without lesion."

Discussion.

Dr. F. C. Wood spoke of an autopsy which he had seen at St. Luke's Hospital, during which a brain tumor was found which had given few symptoms. The case was that of a woman, apparently perfectly healthy, who had been in the hospital for about a week, and had been given large doses of acetanilid and sulphonal. She died very suddenly, it was supposed from the effect of the drug. She had been very nervous and had complained of headache; nothing else had been noted. At autopsy the tumor, a large glioma, was found in the left parietal region.

THE DIFFERENCES NOTED BETWEEN HUMAN AND BOVINE TYPES OF TUBERCLE BACILLI: SUGGESTIONS FOR THE SIMPLEST METHODS OF DIFFERENTIATION.

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The following work was undertaken to determine the type of bacillus in different forms of tuberculosis. It has been done under Dr. Park's direction with the aid of several other workers to carry out the technical part of the investigation. A full report of the details of the work and those concerned in it will be published later. About six hundred cultures have been studied. These were from four hundred and twenty-eight cases of tuberculosis in man, and thirty-one specimens of bovine origin.

Methods of Isolation:—No direct cultures were attempted. All material was injected into guinea-pigs which were allowed to live from three to five weeks. At the end of this period the tuberculous organs were removed and thoroughly minced with knife and forceps. This minced tissue was then smeared over the surface of the culture tube. This mincing and rubbing of the material over the culture media we have found preferable to simply cutting the tissue, laying it on the surface of the media and subsequently moving to spread the growth as advised by Theobald Smith. The second opening of the tube, a fruitful source of contamination with moulds, is avoided ,and the final growth is equal or greater in amount.

Methods of Cultivation:—Egg media of two types have been used for isolation and further cultivation. The ease and simplicity of preparation and the uniformity of the final product make other media for isolation unnecessary.

The egg media were made as follows: Plain egg (Dorset); the whole egg was mixed with 10 per cent. by weight of water and inspissated at 70° C. for two hours in a closed moist chamber. A few drops of sterile water were finally added to each

tube to provide the necessary moisture. Glycerin egg (Lubenau) consisted of ten eggs mixed with 200 c. c. of 5 per cent. glycerin bouillon, 1.5 per cent. acid to phenolphthalein and coagulated as above. Eggs were secured which were quite fresh.

Other media, as glycerin agar, glycerin bouillon and glycerin potato were also used to determine the cultural characteristics and for further cultivation. The glycerin potato has been the most uniformly successful as a stock medium for further cultivation. The cotton stoppers in each case with or without dipping in paraffin were pushed down the tube, which was then closed with a tight-fitting charred cork and incubated in the inclined position.

Nearly all the cultures so far isolated have fallen into two distinct groups, eugonic (luxuriant) and dysgonic (sparse). This difference is noticed on plain egg, but to a less marked degree than on glycerin egg. Here the influence of glycerin on the growth of the two types, as noticed by several investigators and carefully elaborated by Cobbett, causes a wide gap in the amount and rapidity of growth of the two types. The dysgonic type commonly fails to grow in glycerin egg from animal tissue and if transplanted from plain egg after the first or second generation grows but sparsely. The growth consists of thin, flat spreading colonies 1 to 2 mm. in diameter, or of a confluent thin, flat, slightly granular, non-pigmented layer, which is usually moist. The eugonic type, on the other hand, grows luxuriantly in most instances on glycerin egg, directly from the animal tissue and in every case in the second and third generation. The growth is confluent, raised, and crumpled, or coarsely verrucose and dry, and, in practically every case, shows a pink pigmentation in early generations. The pink color depends on certain unknown differences in the different batches of media.

The other media used, as glycerin agar, potato, and bouillon, we have found mainly of value as corroboration of the above differences for the following reason: The eugonic strains grow luxuriantly upon these media usually in the second generation and with few exceptions in the third and fourth. The dys-

gonic strains, on the other hand, usually fail to grow in the early generations, or if they do the growth is very slight.

The differences in the growth of the two types as given apply only to the first few generations and to cultures which are three weeks old, the rapidity of growth as well as the amount being an important factor. In later generations and with longer periods of incubation the differences are not so extreme, and in a few cultures the gap is closing.

The above description and division into distinct and widely separated types of growth is true of the majority of cultures. There are, however, as would be expected in such closely related varieties of the same species of organism, a smaller number of cultures which tend to bridge the gap between these diverging cultural types. At the present time the following classification seems warranted, though further study may make some changes necessary.

Human Type: Group 1.—Glycerin egg: Grade 7 to 8 in the first three generations and pigment on glycerin egg mixtures in the early generations. Glycerin potato: Grade 6 to 8 in the first four generations.

Group 2.—Glycerin egg: Same as Group 1. Glycerin potato: (a) Less than Grade 6, or (b) negative or slight, in first four generations.

Group 3.—Glycerin egg: Grade 4 to 6 with pigment slight or absent in first three generations. Glycerin potato: Grade 3 to 5 in first four generations.

Group 4.—Glycerin egg: As in Group 3. Glycerin potato: Negative or practically so in first four generations.

Group 3 and 4 show no marked increase in amount of growth in the first ten generations.

Bovine Type:—Group 1.—Glycerin egg: Grade 0 to 3 for first three generations; negative or slight, directly from the animal tissue and growth remaining sparse for at least ten generations. Glycerin potato: Slight or negative in the first four generations.

Group 2.—Glycerin egg: Grade 2 to 4 for first three gen-

erations. That is, the growth is better than Group 1, and is usually positive directly from the animal tissue, but not increasing very much in amount in the first ten generations. Glycerin potato: Slight or very moderate growth in the first four generations.

Group 3.—At first as in Groups 1 and 2 on both glycerin egg and potato, but rapidly increasing in amount of growth in the first ten generations reaching Grade 6 or even higher, and showing some pigmentation on reaching the higher grades.

In explanation of the above grouping, the grades of growth refer to the total amount as judged by inspection of the cultures. In each case the best growing tube of any set was considered the typical one. Nine grades were arbitrarily selected; thus: Grades 1 to 3, sparse; Grades 4 to 6, moderate; and Grades 7 to 9, vigorous. The reason for the selection of glycerin egg has already been spoken of. Besides albuminous media of this type we wished also to select some media which would contain no coagulable albumins to serve as a more rigorous index of the saprophytism of the two types. After numerous trials, potato was found to be the most uniform in its results. In some of the earlier cultures before this routine was established other media as glycerin agar and bouillon were used in the early generations, and in these few instances we shall consider them as falling into one of the groups as though potato had been used, as they seem to be absolutely typical in other respects.

When one studies this grouping of the two types there is apparent a gradual graduation from one type to the other. Of the human types no one could mistake Groups 1 and 2 for bovine cultures, nor could they mistake Groups 1 and 2 of the bovine type for human cultures, if they had handled even a few cultures on these media. As to Group 3 of the human type, the difference is still sufficiently marked to make a statement as to type on inspection of the tubes of the first few generations. When Group 4 is reached differentiation is very difficult on these two media, though the fact that the growth reaches its upper limit at the very start points strongly to the human type. Other

media may add some information in these cases, as for instance the ability to grow from glycerin egg on to glycerin bouillon which has been added to the tube, a phenomenon we have not seen in any early bovine culture. Group 3 of the bovine type has been differentiated without exception by the characteristics of the early generations, though one must admit that when the first culture of this type was encountered we considered the possibility of error, but further observation has shown that the early generations, as we thought, are the diagnostic ones. After a few more generations the distinctions in human Groups 3 and 4 and bovine Group 3 are lost and the last tends to outstrip the other two in vigor of growth. These three groups then tend to bridge the gap and form a complete series of types.

This division into two types according to the cultural characteristics has been uniform with the results of animal inoculations, and in practically every case the rabbit virulence has been correctly predicted from the cultural characteristics.

The following is the method we have used in testing the virulence on rabbits: The bacilli are removed from the surface of a subculture which is less than one month old and the excess of moisture is removed by gentle pressure between sterile filter paper before the mass is weighed. The culture medium used for these subcultures, with a few exceptions, has been glycerin egg, and there have been added to the tubes, after coagulation of the medium, a few drops of glycerin bouillon. The growth immediately above the fluid can be readily and perfectly emulsified. After weighing, the bacterial mass is emulsified in physiological salt solution, which is added in such amount that 1 c. c. of emulsion represents 1 mg. of organisms. The dysgonic viruses in many instances afforded too little growth for weighing. An emulsion was, therefore, made and compared with a known emulsion from a eugonic virus, the comparison consisting in the equal ease with which print could be read through each emulsion. A slight amount of saline solution was then added, in order that the error, if any, might fall upon the small side. The amount of fluid inoculated was always 1 c. c. The intravenous

route was decided upon as the most suitable for our purpose, and it has been adhered to throughout the experiment. The dose used has been 1 mg. or 0.01 mg.

It soon became evident that we had to deal with two varieties of organisms, one of which far exceeds its fellow in virulence. An animal inoculated intravenously with 1 mg. or with 0.01 mg. of bacilli of the bovine type soon becomes emaciated, the fur loses its lustre, respiration becomes labored, and death occurs with the larger dose in from seventeen to thirty-eight days with an average period of twenty-one days, and with the smaller dose in from twenty-five to seventy-eight days, with an average of forty-four and six-tenth days. Autopsy reveals extreme emaciation and, except in rare instances,* a generalized tuberculosis involving almost every structure in the body. The lesions in the lymph nodes, and of these particularly the inguinal and the axillary, are almost pathognomonic. The nodes are enlarged, congested and studded with minute caseous areas. The spleen is three or four times its normal size and riddled with tubercles, and the kidneys show on their surfaces an average of from six to ten tubercles each. The liver very commonly contains macroscopic tubercles. The lymph nodes of the abdomen are usually enlarged, as are those of the thorax, and commonly show small tubercles which may be caseous. The lungs are always largely consolidated, and are literally a mass of tubercles. Tubercles on the heart muscles are not infrequent. The duration of the illness is shortened by increase of the dose, but is also influenced by the individual resistance of the animals, nor does it seem that old rabbits are much more resistant than young ones.

Separated from such viruses by a wide interval of virulence are the eugonic viruses. Those never cause generalized progressive lesions in doses of 0.01 mg. or even 1 mg., and a rabbit so inoculated will usually live until carried off by an in-

*There were four of these rabbits in which the lesions were localized chiefly in the lungs. The same cultures used in the smaller dose produced generalized tuberculosis.

tercurrent malady. The appearance at autopsy is in striking contrast to the findings described in the preceding paragraph. The animal will be found well provided with subcutaneous and abdominal fat, and the lymph nodes, with few exceptions, are normal in size and appearance. The appearance of the lungs varies with the length of time that has elapsed since inoculation. About six weeks after injection they are pretty extensively involved, for at this point, of course, many of the inoculated organisms are arrested. But that the lesions under discussion are regressive is shown by the fact that many animals have gained weight, as well as by our observation that in rabbits autopsied after an interval of three or four months, the lungs are pink, well collapsed, and with tubercles only sparingly distributed throughout them. Almost always there are a few tubercles in the kidneys. The spleen is of normal size, and it is very rare that a tubercle may be found on its surface. Tubercles in the heart muscle are quite uniformly absent. Occasionally after a long interval, there occurs a chronic caseating tuberculous lesion such as orchitis, osteomyelitis, adenitis, mastitis, or large cold abscess.

As in the cultural characteristics of the two types so in the widely divergent types of virulence for rabbits, as described, are included the great majority of cultures. Here also, there are some intermediate grades of virulence apparent. The viruses which show this variation in virulence have not been sufficiently studied as yet to warrant any subdivision of groups as in the cultural characteristics. At the present time, it is impossible to say how far there will be a tendency to fill the gap between the two extremes of virulence. We can say, however, that these variations have not given us any reason to doubt the reliability of the virulence test in rabbits as a means of differentiation when the test is properly controlled by repetition where any variation occurs in one or more rabbits of a series.

Wherever any intermediate characteristics of cultures or rabbit virulence have occurred, the combined evidence of both has left no doubt as to the type of organism.

In a series of selected cultures we have tested the virulence on young calves. Fifty milligrams of culture emsulsified in 5 c.c of normal saline was injected subcutaneously in the neck. Here again the cultures have fallen into two distinct groups. The human type caused only a local lesion or at most an extension into the neighboring lymph nodes and had no effect on the health of the calf. The bovine type on the other hand produced an acutely fatal generalized tuberculosis. Varying from these two extremes was the result in one calf receiving a bovine virus, where a chronic and apparently regressive generalized tuberculosis was caused.

The above description has rather accentuated the variations from the widely separated characteristics of the two types. We have done this to show plainly the fallacy of working with a few cultures and then questioning the existence of two distinct types of tubercle bacilli. This would probably be the result if an investigator should happen upon several variants in a small series of cultures. On the other hand if all cultures were absolutely typical of the two extremes his conclusions would be diametrically opposite. In neither case would his results be true. Our own conclusions have been modified from time to time, the earlier work pointing to wide divergence only, the latter work showing variations from these results. This is really what one would expect from analogy with other bacteria, viz., that in two closely related types of organism there is not a wide gap, but rather two norms around which the two types vary, these variants in no way causing any doubt as to the existence of the two types.

From the foregoing descriptions it is evident that the simple observation of the amount of growth forms a reliable index of the type in the great majority of cultures. We would suggest the following simple routine for differentiation using the media given. First, isolation using egg and glycerin egg, then transplanting for three generations at three week intervals on these media plus glycerin potato, the egg being used because growth may fail on the other media. All vigorously growing cultures

would immediately class themselves as human, all feebly growing cultures as bovines. This would dispose of nearly all cultures. The remaining cultures, if any, showing variations from these extreme types should then be tested in rabbits giving an intravenous dose of 0.01 mg. If the rabbit survives, kill after a period of fifty or sixty days, and the generalization or non-generalization will settle the question of type. As far as the time limit is concerned we are still experimenting. A safe rule, however, would be to insist that rabbits survive fifty days and then show no generalization before the culture be considered of the human type. Early generations should be used for inoculations. It is necessary to say that the utmost care be taken that the cultures be pure both by microscopic examination and transplants on ordinary media. A contamination that escapes one may cause a human strain to simulate the bovine type of growth.

The above routine if carefully followed gives all that is apparently necessary to separate the types, although it gives a limited amount of information as to the characteristics of a culture. There is always the possibility of a strain giving atypical results in both culture and rabbits. These cases are in the great minority and will be noted if above steps are carried out, and further observations can then be made. Another possible factor is avian tuberculosis. There does not seem to be any reason for considering this in view of the evidence that has been advanced.

Discussion.

Dr. Alfred F. Hess agreed that tubercle bacilli could well be divided into two types and that differentiation between the human and the bovine bacilli was possible by the first cultures. It very rarely happened that a mistake was made. In cases where a misjudgment might be made, as where the bovine type grew particularly well, or the human cultures particularly poorly, a rabbit inoculation would be sure to show whether the culture were bovine or human. A great many observers did not

accept this sharp differentiation into human and bovine type; certainly there were some human types which resembled the bovine, and some bovine which resembled the human; but by taking cognizance of the cultural types and virulence, there were no cultures which could not be placed in one group or the other. It was possible that tubercle bacilli could be altered somewhat in their cultural characteristics and virulence in the human body; that a bovine bacillus in the human body for a number of years might grow more luxuriantly and lose some of its virulence. This had never been proved, though it seemed to be the only way to account for some bovine strains which resemble human strains. Dr. Krumwiede had a case where the human and the bovine type were found in one individual. This finding was difficult to explain, as, for example, where the human type appeared in the spleen, and the bovine type in the mesenteric glands, or vice versa. That had given occasion to some for saying that one type might change into another, and was certainly difficult to understand. Dr. Hess spoke of two important facts in the differentiation of the bacilli: First, no case had as yet been reported in which bovine tubercle bacilli were isolated from the lungs; all cases of pulmonary tuberculosis had been of the human type. Second, all cases of bone and joint tuberculosis, with one exception, had been of the human type. So that if one got a primary pulmonary tuberculosis he was pretty safe in saying that the bacillus was of the human type. Yet many believed that bone and joint tuberculosis were of bovine origin. All other tuberculoses had been found to be either human or bovine and could not be charged to one group.

Dr. Krumwiede said that in one case the bovine bacilli had been isolated from the cerebrospinal fluid, and the human bacilli from the mesenteric glands.